**Remarks**

The rejections of claims 1, 5, 8-14, 21-23 and 75-77 under 35 USC 103(a) as unpatentable over Lacroix in view of Mitra and further in view of Havel have been maintained.

Applicants reiterate their contention that cited references do not teach or suggest determining a tertiary structure of a protein by applying physical distance constraint information associated with cross-linking to the a set of candidate three-dimensional conformations to rank and select one or more conformations.

Specifically, Applicants submit that none of the cited references, alone or in combination, teaches 1) proposing a set of candidate three-dimensional conformations (for the protein's primary sequence) and then selecting from this set one or more conformations, or 2) applying distance information associated with cross-linking to the candidate set of 3-D conformations to rank and select the one or more conformations.

Applicants address the specific remarks related to the references that were raised in the previous two Office Actions.

**Lacroix**

Lacroix is relied upon for providing a candidate set of 3-D conformations from which one or more conformations is selected. Rossi, referred to in Lacroix, is cited in particular for showing a candidate set of 3-D conformations.

Lacroix and Rossi relate to domain-domain placement of relatively large protein domains. Applicants note that both Lacroix and Rossi are discussed in Applicants' specification at page 10, lines 23-26 and further discuss both references below.

Both Lacroix and Rossi describe studies of the γ-B component of multidomain proteases. Lacroix describes a study of the γ-B component in the C1r multidomain

protease, which contains a $(\gamma\text{-B})_2$ dimer; Rossi describes a study of the γ-B component of the C1s multidomain protease, which contains a γ-B monomer. Both references use cross-link information to propose an assembly of relative large domains or modules of the γ-B component, each domain or module being pre-analyzed to define its conformation.

Each γ-B monomer is a single-chain polypeptide comprising a tandem repeat of so-called "complement repeat protein modules" (or CCP modules), a 15-residue intermediary segment (IS), and a serine protease (B) domain (see Lacroix, abstract; Rossi, abstract). Lacroix and Rossi describe a two-step process in determining the structure of the γ-B component.

First, each of the CCP IV, CCP V, and B modules are pre-analyzed to define its conformation by homology modeling (Lacroix, abstract and page 6272; col. 1; Rossi, abstract and page 7313, col. 1). Second, after the conformations of the modules are determined, cross-linking information is used to position the modules with respect to one another, thereby determining the structure of the component.

Lacroix shows this assembly of the γ-B monomer and the $(\gamma\text{-B})_2$ dimer of the protein region by applying cross-link information to position the modules (of fixed conformation) with respect to each other (page 6278, cols. 1-2). Figs. 9-11 show the results of positioning these modules; Fig. 9 shows CCP modules IV and V assembled to form the γ-B monomer; Fig. 10 shows these modules assembled to form a $(\gamma\text{-B})_2$ dimer; and Fig. 11 shows an alternate view of the same $(\gamma\text{-B})_2$ dimer structure shown in Fig. 10.

Similarly, Rossi describes assembly of the γ-B monomer in the C1s region by determining the relative positions of CCP modules IV and V, the IS and the B domain (Fig. 9; pages 7316-19).

The Examiner appears to be taking the position that these assembled structures depicted in Figures 9-11 of Lacroix are the "candidate set of 3-D conformations" recited in the claims (page 4, fourth full paragraph in the 8/05 Office Action).

Applicants submit that these figures do not show a "candidate set of conformations" from which one or more are selected to determine a tertiary structure. Rather, as discussed above, these show the already assembled or determined structures of the ($\gamma$-B) monomer and ($\gamma$-B)$_2$ dimer.

The Examiner also points to Rossi as showing a candidate set of 3-D conformations:

> "Lacroix discloses that the homology modeling is similar to that of Rossi. Rossi et al disclose a threading technique by where a set homologous three-dimensional structures is used as a reference template, sequence of proteins are aligned and the candidate structure is identified by comparing said structure to the reference set. (Rossi et. al, page 7313...columns 1-2)."

The cited portion of Rossi describes a program "O" to construct homology based models of various individual modules of the $\gamma$-B monomer and then assembly of the $\gamma$-B monomer using information provided by chemical cross-linking. Rossi and Lacroix use a homology modeling technique to separately characterize each of the modules (the B chain and two CCP modules).

Specifically, the program "O" uses a set of homologous three-dimensional structures as a reference template. The module to be modeled is aligned on the sequences of the reference set, with the reference structure exhibiting the fewest insertions and deletions to the module used as the primary template for further modeling of the module. Thus, both Rossi and Lacroix mention only conventional homology modeling for the purpose of building models of the individual modules. While they may have been built using multiple three-dimensional structures of pre-solved proteins, there is not teaching or suggestion of applying the "physical distance constraint information associated with the identified cross-link fragments" to choose a three-dimensional structure from among these multiple structures. Rather, only the number of insertions and deletions is used to select the reference structure to be used as a template.

Rossi does use cross-link information to locate and assemble previously defined modules into the overall $\gamma$-B structure. See the discussion at pages 7318 and

7319. See also the discussion at page 6278 of Lacroix. Thus, cross-linking information in Lacroix/Rossi is used to assemble these modules – not to rank and select one or more of said three-dimensional conformations from a set of candidate three-dimensional conformations as claimed.

Thus, Applicants submit that Lacroix does not teach or suggest 1) applying distance information to a candidate set of 3-D conformations or 2) using distance information associated with cross-linking to rank and select a conformation to thereby determine the tertiary structure.

**Mitra**

Mitra, also discussed in Applicants' specification at page 2, lines 2 and 3, teaches reagents for cross-linking proteins. As discussed in previous Amendments, Lacroix teaches producing intra-monomer cross-links, and then using this cross-link information to position the domains of the γ-B monomer with respect to one another, thereby constructing a three-dimensional model of the γ-B monomer.

Combining the cross-linking reagents taught in Mitra with the method of protein structure determination of Lacroix would not result in the claimed invention. Rather, it would result in using the cross-linking reagents taught in Mitra to produce intra-monomer cross-links (within a single γ-B monomer) and inter-monomer cross-links (between the individual residues of the separate γ-B monomers in a $(\gamma\text{-B})_2$ dimer) as taught in Lacroix.

The Examiner responded to this argument in present Office Action by stating that:

> "This is not persuasive because there is nothing in the instant claims that precludes providing intra-monomer or inter-monomer cross-links. The point of Lacroix is to teach three-dimensional modeling of a protein using cross-linking as instantly claimed."

While it is true that nothing precludes this, the instant claims require applying the cross-link information "to the candidate three-dimensional conformations". Because Lacroix teaches applying cross-link information to position the modules with respect

to each other (and to not to the assembled 3-D conformations or to determine the structure of the individual modules), a reasonable combination of Mitra with Lacroix would also result in applying cross-linking information in this manner. As the individual modules are not a set of proposed 3-D conformations for the tertiary structure of the protein, but pieces of the component to be assembled, the combining Mitra with Lacroix does not meet this limitation.

At least because there is there is no teaching or suggestion of selecting one or more conformations from a candidate set of conformations, or of applying distance information associated with cross-linking to candidate conformations to select a conformation in Mitra, it does not cure the deficiencies of Lacroix.

**Havel**

Havel is relied upon for distance information as well as providing a candidate set of 3-D conformations. Specifically the Examiner cites page 79, paragraph 2, in writing:

> "Havel states that the residues can be cross-linked in the laboratory and that the data is capable of imposing significant constraints on the range of possible structures when the number of distances measurable is comparable with the residues in the chain. Conformations consistent with this information were produced by constraining each residue..." (present Office Action, page 5)

Applicants submit that Havel also does not teach or suggest 1) applying distance information to a candidate set of 3-D conformations or 2) using distance information to rank and select a conformation to thereby determine the tertiary structure.

Havel, also discussed in Applicants specification at page 3, lines 10-20, is concerned with testing the efficacy of using constraint information to generate structures. The authors conclude by making generalizations about how one can determine tertiary conformations of macromolecules by considering interresidue contacts. The contacts are derived from various residue positions in known protein structures. Most of these do not involve crosslinks of any type. In the actual work

performed by the authors, crosslinks are limited to Native cystine -S-S- crosslinks. Crosslinks imposed as part of a physical process were not considered. Therefore the relevance of the Havel reference to the pending claims is extremely limited. Note that claim 1 recites "imposing physical distance constraints between residues of the protein by cross-linking the protein" and "fragmenting the protein." To the extent that Havel et al. may speculate about use of imposed crosslinks, this speculation fails to suggest 1) applying distance information to a candidate set of 3-D conformations or 2) using distance information to rank and select a conformation to thereby determine the tertiary structure.

Generally Havel describes using a set of basic geometric constraints to generate structures of two proteins. Ten structures are generated for each protein in this manner (page 75). Mean quantities of the ten structures are calculated to provide information about the efficacy of using the constraints to construct structures. Specifically, the mean rms difference of the 45 different possible pairs of the structures is found ($E_s$) as a measure of volume in conformation space. In addition, the mean rms distance between each of the ten structures and the x-ray crystal structure ($E_x$) is found as a measure of distance to the crystal structure in conformation space. The process was repeated for different sets of distance constraints. $E_s$ and $E_x$ were then used to evaluate the efficacy of each set of constraints in generating structures. Applicants note that the "best case" language cited in the August 2005 Office Action does not refer to selecting a conformation, but to the best case scenario of interpreting the results of the study ("Our results should be taken as an upper bound (best case) on the effectiveness of such studies in restricting the range of allowed conformations") (page 74).

Thus, while Havel does teach generating multiple structures using distance constraints, Havel does not teach or suggest applying distance the constraint information to these generated structures. Rather, Havel uses constraints to generate the structures. Havel also does not teach or suggest selecting one or more structures from amongst the generated structures. Rather, information about all the structures are averaged and compared to the x-ray structure to determine the effectiveness of the particular constraints used to generate the structures. Nor does Havel suggest any

way that the constraint information may be used to rank and select one or more of the generated structures.

For at least these reasons, it is submitted that Havel does not cure the deficiencies of Lacroix.

**Conclusion**

For at least the reasons discussed above, the combination of references does not suggest the use of cross-linking and subsequent analysis as claimed to determine a tertiary structure of a polypeptide. Applicants believe that these arguments fully address all of the issues raised in the Final Office Action. If the Examiner wishes to telephone the applicants representative concerning any matter pertaining to this case, the Examiner is cordially invited to do so at the telephone number set out below. The Commissioner is hereby authorized to charge any additional fees to Deposit Account 500388 (Order No. UCSFP001).

Respectfully submitted,
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